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A. C. TRUE, Director.

EXPERIMENTS

ON

THE METABOLISM OF NITROGEN, SULPHUR, AND PHOSPHORUS IN THE HUMAN ORGANISM.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Office of Experiment Stations,
Washington, D. C., October 1, 1902.

Sir: I have the honor to transmit herewith a report on experiments on the metabolism of nitrogen, sulphur, and phosphorus in the human organism, carried on by H. C. Sherman, Ph. D., instructor in analytical chemistry at Columbia University, New York, in cooperation with this Department. The investigations were conducted under the immediate supervision of Prof. W. O. Atwater, chief of nutrition investigations, and form a part of the investigations on the food of man conducted under the auspices of this Office. Doctor Sherman's investigations have for their special object a study of the cleavage of protein, with reference particularly to the way in which this nutrient serves for building tissue and as a source of energy. The results given herewith constitute a progress report.

The report is submitted with the recommendation that it be published as Bulletin No. 121 of this Office.

Respectfully,

A. C. True,
Director.

Hon. James Wilson,
Secretary of Agriculture.

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INTRODUCTION.

Most of the digestion experiments heretofore reported in connection with the nutrition investigations of this Department have included the determination of the balance of income and outgo of nitrogen, while in those carried out in the respiration calorimeter the balance of carbon, hydrogen, and energy are likewise determined. It is believed that in many cases the determination of income and outgo of sulphur and phosphorus will add considerably to the interest and value of these investigations.

The sulphur or phosphorus balance, like the nitrogen balance, may be found by comparing the amounts ingested in the food with those eliminated through the kidneys and intestines. So far as is known no phosphorus and only traces of sulphur escape in the form of volatile compounds, and the quantities of sulphates and phosphates in the perspiration are so small that they may probably be neglected, unless in exceptional cases.

METABOLISM AND ELIMINATION OF SULPHUR.

Small quantities of sulphates occur in foods and in some waters. By far the greater part of the sulphur of the food enters the body in organic combination, in proteids or albuminoids. When proteid matter is oxidized in the body most of the sulphur is burned to sulphuric acid, the greater part of which appears in the urine as normal inorganic sulphates. A smaller part of the sulphuric acid (in health usually about one-tenth) is found in the form of ethereal sulphates, i. e., combined with organic radicles, the latter being usually regarded as derived chiefly from intestinal putrefaction of proteids. Such putrefaction may give rise to the formation of hydrogen sulphid, which may either appear as sulphids, chiefly of iron and the alkali metals, in the feces, or may be absorbed into the system, or may to some extent escape with the intestinal gases. The total sulphates of the urine may readily be determined by precipitation as barium sulphate after boiling the urine with hydrochloric acid to set free the sulphuric acid in "ethereal" combination. Not all of the urinary sulphur, however, exists in the form of sulphates. About 15 to 20 per cent is

usually found in less completely oxidized forms, this portion being called "unoxidized" or "neutral" sulphur, to distinguish it from the fully oxidized sulphate-sulphur. The existence of sulphur in other forms than sulphates in the urine was discovered by Ronalds at Giessen in 1846,[a] but was first brought into prominence by Bischoff and Voit in 1860.[b] Several compounds have been described as contributing to the "neutral" sulphur of the urine. The taurin of the bile is held to be largely reabsorbed from the intestines and eliminated through the kidneys. If taurin be fed directly the amount of neutral sulphur in the urine increases, according to Salkowski,[c] and in experiments upon a dog with a biliary fistula the neutral sulphur was found to decrease, but did not entirely disappear.[d] Among other sulphur compounds which have been found in the urine may be mentioned sulphocyanids, originally derived from swallowed saliva,[e] thiosulphates, small quantities of cystin,[f] of mucin, and occasionally of hydrogen sulphid. Abel[g] has described a body which yields ethyl sulphid, and it is probable that other compounds remain to be discovered, since the quantities of the above compounds believed to exist in normal urine are not sufficient to account for all of the neutral sulphur found. According to Spiegel[h] the appearance of cystin and hyposulphites in the urine points to a condition of diminished oxidation, since these compounds though constantly formed in the body are not normally end products of metabolism.

The following quotations from recent text-books (which are given in chronological order) are believed to fairly represent the present general teachings in regard to the significance of the sulphur metabolism and its relation to the metabolism of nitrogen.

Halliburton[i] says:

> The sulphuric acid of the urine is in part combined as ordinary sulphates, in part as ethereal sulphates. It is derived to a small extent from the food, but chiefly from the metabolism of proteids, the amounts of sulphuric acid and urea in the urine running parallel.

According to Hammarsten:[j]

> The sulphuric acid of the urine originates only to a very small extent from the sulphates of the food. A disproportionally greater part is formed by the burning

[a] Falck's Beiträge zur Physiologie, Hygiene, etc., p. 102.

[b] Gesetze der Ernährung des Fleischfressers, pp. 279–284, 302–303.

[c] Cf. Lusk, American Text-book of Physiology, Vol. I, p. 507.

[d] Kunkel, Arch. Physiol. [Pflüger], **14** (1887), p. 353.

[e] Leared, Proc. Royal Soc. London, 1870, pp. 16, 18; I. Munk, Arch. Path. Anat. u. Physiol. [Virchow], **69** (1877), p. 354.

[f] Goldmann and Baumann, Ztschr. Physiol. Chem., **12** (1888), p. 254.

[g] Ztschr. Physiol. Chem., **20** (1894), p. 253.

[h] Arch. Path. Anat. u. Physiol. [Virchow], **166** (1901), pp. 364–371; abs. in Jour. Chem. Soc. [London], **82** (1902), No. 471, II, p. 93.

[i] Schaffer's Text-book of Physiology, Vol. I, 1898, p. 79.

[j] Text-book of Physiological Chemistry, trans. by J. A. Mandel, 1898, p. 515.

of the proteids containing sulphur within the body, and it is chiefly this formation of sulphuric acid from the proteids which gives rise to the previously mentioned excess of acids over the bases in the urine. The quantity of sulphuric acid eliminated by the urine amounts to 2.5 grams H_2SO_4 per 24 hours. As the sulphuric acid chiefly originates from the proteids, it follows that the elimination of sulphuric acid and the elimination of nitrogen are nearly parallel, and the relationship $N : H_2SO_4$, is about 4.5 : 1. A complete parallelism can hardly be expected, as in the first place, a part of the sulphur is always eliminated as neutral sulphur, and secondly, because the low quantity of sulphur in different protein bodies undergoes greater variation as compared with the high quantity of nitrogen contained therein. Generally the relationship between the elimination of nitrogen and sulphuric acid under normal and diseased conditions runs rather parallel.

In Novy's[a] opinion:

The proteins of the food and of the tissues constitute almost the sole source of the sulphur containing waste products. A small amount of waste sulphur compounds is eliminated as sulphocyanate by the saliva, gastric juice, etc. Another small portion leaves the body as taurin in the taurocholic acid of the bile. With these exceptions almost all the sulphur resulting from protein disintegration appears in the urine. Inasmuch as the sulphates contain most of the waste sulphur it follows that the total sulphates in the urine furnish an excellent index of proteid disintegration.

According to Ogden:[b]

The total quantity of sulphuric acid in the twenty-four hours' amount of urine of an adult taking a mixed diet is from 1½ to 3 grams, or an average of 2 grams. About one-tenth of the total sulphuric acid is in the form of ethereal sulphates. In general it may be stated that the variation in the quantity of ordinary sulphates eliminated in the urine runs parallel to that of urea.

Lusk[c] states that:

Sulphur is built in the proteid molecule of the plant from the sulphates taken from the ground. It is found in albuminoids, especially in keratin. As taurin it occurs in muscle and in bile, as iron and alkaline sulphids in the feces, as sulphureted hydrogen in the intestinal gas, as sulphate and other unknown compounds in the urine. * * * The total amount of sulphur in the urine runs proportionately parallel with the amount of nitrogen; that is to say, the amount is proportional to the amount of proteid destroyed. * * * When an animal eats proteid and neither gains nor loses the same in his body, the amount of sulphur is equal to the sum of that found in the urine and feces. Sulphates eaten pass out through the urine. They play no part in the life of the cell.

Thus there is general agreement in regarding the sulphur of the urine as essentially derived from katabolism of proteid in the body, so that the quantity eliminated is, like that of nitrogen, an indication of the amount of proteid matter broken down. This agreement in regard to the parallelism of the nitrogen and sulphur excretion is, however, by no means exact, since in some cases the reference is to total sulphur, in others to total sulphates, and in still others to "ordinary" sulphates.

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[a] Physiological Chemistry, second ed., 1898, pp. 194, 195.

[b] Clinical Examination of the Urine, 1900, p. 111.

[c] American Text-book of Physiology, second ed., Vol. I, 1900, pp. 505, 507.

Of the authors quoted, Hammarsten is the only one to call attention to the fact that this parallelism will be affected by the variations in the relative proportions of nitrogen and sulphur in different proteids. That these variations are very large will be seen from a comparison of the accepted analyses of a few representative proteids. Taking, for instance, the elementary analyses recently compiled by Osborne in connection with his discussion of the sulphur in proteid bodies,[a] we estimate from the percentages given that the ratio of nitrogen to sulphur is, in legumin as 46.9:1; in zein, 26.9:1; in edestin, 21.2:1; in bynin, 19.4:1; in gliadin, 17.2:1; and in leucosin, 13.1:1. From this it will appear that the typical proteids of wheat furnish about three times as much sulphur, with a given amount of nitrogen, as the typical proteid of the legumes. The ratio in casein (19.7:1) is about twice as great as in egg albumin (9.6:1). Among the proteid constituents of the body the differences are even greater than among the food proteids just mentioned. In oxyhæmoglobin the ratio is 44.6:1; in myosin, 13.1:1; in serum globulin, 14.3:1; in fibrinogen, 13.3:1; in serum albumin from human exudation, 7.06:1; in chondromucoid,[b] 5.2:1; in tendon-mucin,[b] 5:1, and in osseomucoid,[b] 5:1.

Thus it would appear that the katabolism of sufficient glucoproteid to yield a gram of nitrogen would result in the elimination of about three times as much sulphur as the katabolism of an equivalent amount of myosin, serum globulin, or gliadin, and nearly ten times as much as would come from an equivalent amount of oxyhæmoglobin or of legumin. It is evident, therefore, that the ratio of nitrogen to sulphur in the urine may undergo considerable variation as the result of changes in the kind of proteid given in the food or in the kind of body tissue katabolized in case the protein of the food is insufficient. The interesting investigations of Kolpatcka noted below (p. 13) are based largely on these variations. There is, however, every reason to believe that so long as the diet is uniform, and other conditions normal, the metabolism and elimination of sulphur will be nearly parallel with that of nitrogen; and this seems to be true not only as concerns the twenty-four hours' urine, but usually for shorter periods as well. (See p. 45.)

METABOLISM AND ELIMINATION OF PHOSPHORUS.

Phosphorus enters the body in organic combination in the form of nucleins, nucleo-proteids, lecithin, protagon, and perhaps glycerol-

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[a] Connecticut State Sta. Rpt. 1900, p. 464; Jour. Amer. Chem. Soc., 24 (1902), p. 140.

[b] The ratios given for the glucoproteids are from figures given by Hawk and Gies (Amer. Jour. Physiol., 5 (1901), p. 416. In the case of osseomucoid the average of the later and purer preparations is taken. The figures for tendon-mucin (Chittenden and Gies) have recently been confirmed by Cutter and Gies (Amer. Jour. Physiol., 6 (1902), p. 155. The figures for chondromucoid are from the work of Mörner (Ztschr. Physiol. Chem., 18 (1893), p. 213.

phosphoric acid, but a larger quantity is taken as mineral phosphates in the food. The proportion of phosphorus eliminated by the intestine depends mainly on the nature of the food and the alkalinity of the blood. Herbivora excrete nearly all of the phosphorus with the feces, and in man the amount thus excreted is greatest on a vegetable diet or one rich in lime salts, and may be largely increased by feeding alkaline citrate and calcium carbonate, the first to furnish the more alkaline reaction to the blood and urine, the second to form with the phosphoric acid the insoluble phosphate of lime (Lusk).[a]

The phosphorus of the urine is present chiefly as phosphates of the alkalies, with a much smaller quantity of phosphates of the alkaline earths. A very small proportion is present in organic combination. This has been believed to exist as glycerol-phosphoric acid. Jolly,[b] however, claims to have found in the urine certain peculiar nitrogenous compounds, which retain some mineral phosphate in such intimate association that the phosphoric acid is not precipitated by the usual reagents, and he believes that it is these phosphates and not glycerol-phosphoric acid or any incompletely oxidized form of phosphorus which escapes precipitation by the ordinary methods.

Since the phosphorus of the urine comes so largely from the simple passage through the system of the phosphates taken in the food, it follows that variations in the quantity eliminated are more apt to be connected with the diet than with the metabolism of body material. The idea once held that the quantity of phosphorus eliminated is principally dependent upon the metabolism of nervous tissue was soon abandoned. In this connection Voit[c] stated that the bones contain about 1,400 grams of phosphorus, the muscles about 130 grams, and the brain and nervous system about 12 grams. Moreover, by comparing the loss of weight of different organs in the starving dog, with the changes in the ratio of nitrogen to phosphorus in the urine, he was able to show that the body material katabolized was largely contributed by the bones.

Recent work tends to emphasize the importance of the nucleins and related bodies and to confirm the view that the phosphates found in all the organs and tissues of the body are to a considerable extent in chemical combination with the proteid matter. Thus it is stated that when the body stores proteid a proportionate amount of phosphoric acid is retained for the new protoplasm, while on destruction of proteid the phosphoric acid corresponding to it is eliminated.[d]

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[a] See also the recent work of Paton and his associates (Jour. Physiol., 25 (1900), p. 212), comparing the metabolism and elimination of phosphorus in the dog and in the goat.

[b] Compt. Rend. Acad. Sci. Paris, 127 (1898), 118.

[c] Hermann's Handbuch des Physiologie, vol. 6, pt. 1, p. 80.

[d] Lusk, American Text-book of Physiology, second ed., 1900, Vol. I, p. 575.

The significance of the phosphorus metabolism from the medical standpoint is quite fully discussed by Bergell.[a]

Several investigators[b] have recently studied the urinary excretion of phosphates as influenced by those conditions which are believed to be especially connected with the metabolism of nucleins.

An intimate connection between changes in the phosphorus eliminated and in the katabolism of nucleins is evidently assumed by Dunlop, Paton, Stockmann, and Maccadam in interpreting the results of their investigations of the effects of muscular exertion.[c] In these experiments each subject maintained a uniform diet for seven days, on the fourth of which as much exercise (bicycle riding) was taken as the subject could endure without serious discomfort. In each case the day or days following the exertion showed an increased elimination of nitrogen and sulphur, but only when the subject was in poor training was there a corresponding increase in the elimination of phosphates and of uric acid. From this it was concluded that with the subject in good training only muscle proteid is broken down, while if the subject be in poor training this consumption of muscle proteid is accompanied by the consumption of the material of other tissues which contain nucleo-proteid. In this connection it is interesting to note the observation previously made by Preysz,[d] that the increased elimination of phosphoric acid resulting from walking a given distance (25 kilometers) was considerably greater when the distance was walked at a rapid rate, causing a more intense though less prolonged exertion.

As already stated, the greater part of the phosphorus eliminated comes from the phosphates of the food. When, however, the diet is uniform, a variation in the phosphorus elimination must be taken as showing some change either in body metabolism or in the condition of the body with reference to its store of phosphates. Whether or not the connection between urinary phosphates and the katabolism of nucleins is as intimate as some investigators seem to assume, it is evident that the study of the phosphorus balance may give valuable information which could not otherwise be obtained regarding the nature of the changes taking place in the body.

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[a] Fortschr. Med., 16 (1898), p. 1. Bedeutung der Phosphorsäure in menschlichen und thierschen Organismen. Inaug. Diss., Berlin, 1898.

[b] Moraczewski. Arch. Path. Anat. u. Physiol. [Virchow], **151** (1898), p. 22; Milroy and Malcolm, Jour. Physiol., **23** (1898), p. 217, and **25** (1899), p. 105; White and Hopkins, Ibid., **24** (1899), p. 42; Loewi, Arch. Exper. Path. u. Pharmakol., **44** (1900–1901), p. 1; abs. in Jour. Chem. Soc. [London], 78, 1900, II, p. 417.

[c] Jour. Physiol., **22** (1897–98), p. 68.

[d] Ungar. Arch. Med., 1 (1892–93), p. 38; reviewed in Arch. Physiol. [Pflüger], **54** (1893), p. 21.

PREVIOUS WORK ON THE COMPARATIVE METABOLISM OF NITROGEN, SULPHUR, AND PHOSPHORUS.

The course of the elimination during the day.—Considerable attention has been given by different investigators to the course of the elimination of nitrogen and of phosphorus during the day. The recent work of Rosemann[a] on nitrogen and of his pupil Roeske[b] on phosphorus may be especially noted. Unfortunately such studies have usually been made upon only one element at a time. In some recent experiments carried out in the laboratories of Wesleyan University[c] the course of elimination of nitrogen, sulphur, and phosphorus has been observed simultaneously, the urine being collected in the three-hour periods during the day with one nine-hour period at night. The rates of elimination of nitrogen and sulphur were found to run nearly parallel, rising and falling twice during the day and reaching a minimum during the night. The fluctuations, though quite regular, were not very great, the highest rate of elimination found during the day being usually about one-fourth greater than the average rate for the nine hours of the night. The elimination of phosphorus, on the other hand, did not run parallel with that of nitrogen and sulphur, and the fluctuations, though less regular, were considerably larger, the maximum rate of elimination being two to three times as great as the minimum. Moreover, the minimum rate of elimination of phosphorus was reached not during the night, but at some time in the forenoon, usually from one to three hours, but sometimes from four to six hours after rising.

Comparative metabolism during periods of a day or more.—Many metabolism experiments have been made in which nitrogen and phosphorus were determined and a smaller number in which sulphur was also included. Several of these investigations will be referred to later in connection with the discussion of the results of experiments here reported. The investigations of Kolpatcka[d] are, however, so suggestive that they should be mentioned here. The subjects were in all cases dogs, and the object of the work was to learn the real source of the nitrogen in the urine—to determine whether it is derived directly from the protein of the food, from protein stored in the body, or from actual proteid tissue—and further, to study the nature of the stored protein.

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[a] Arch. Physiol. [Pflüger], **66** (1896), p. 343.

[b] Ueber den Verlauf der Phosphorsäure Ausscheidung beim Menschen. Inaug. Diss., Greifswald, 1897.

[c] Sherman and Hawk, Amer. Jour. Physiol., 4 (1900). p. 25, and unpublished results by Atwater and Hawk and by Hawk and Chamberlain. These experiments are more fully described in connection with the discussion of "lag" on p. 36.

[d] Phiziologicheskii Sbornik. A. I. and V. I. Danilevski, editors. Kharkov, 1888, Vol. I, p. 53; abs. in U. S. Dept. Agr., Office of Experiment Stations Bul. 45, pp. 308, 322.

Kolpatcka endeavored to solve these problems by comparing the ratios of phosphoric acid to nitrogen and of sulphur to nitrogen in the food consumed and in the urine. The ratios found in the foods used were as follows: In meat, $P_2O_5:N::1:7.3$; $S:N::1:15.6$. In gelatin, which contains no P_2O_5, the ratio is as follows: $S:N::1:22.5$. In whites of eggs, $P_2O_5:N::1:47.6$; $S:N::1:9.8$. In yolks of eggs, $P_2O_5:N::1:1.8$. Knowing the ratios of these elements in the food and in the urine during partial or complete fasting, it was held to be possible to judge whether the nitrogen in the urine for any particular period came from the food consumed, from stored protein, or from actual body tissue. Thus on a meat diet the ratio of $P_2O_5:N$ in the urine was nearly the same as in the food, and it was concluded that the excreted nitrogen came directly from the food. During a period of fasting following the meat diet the relative proportion of phosphorus excreted gradually increased until the fifth day, after which the ratio was nearly constant, $P_2O_5:N::1:4$ (about). A similar change in the ratio was found after a change from meat diet to a diet of fat and starch. These results are held to show that when the supply of protein is cut off there follows a katabolism, first of protein simply stored from the previous diet and not yet organized, then of protein from body tissue, this last being the sole source after the fifth day and yielding a relatively large proportion of phosphorus. The increased proportion of "earthy" phosphates led to the belief that some of the "tissue protein" came from the bones, a conclusion reached several years ago by Voit. (See p. 11.)

On passing from a meat ration to a ration of white of egg there was a diminution of phosphoric acid and an increase of sulphur. The ratios, however, varied considerably, and a relatively large amount of phosphoric acid in the first days was attributed to a destruction of some body protein. In passing from a meat to a gelatin ration the amount of nitrogen in the urine increased, while the amount of phosphoric acid decreased but did not entirely disappear, thus giving additional evidence that gelatin alone can not prevent the breaking down of protein tissue. In several cases the experiments were repeated with substantially the same result. So far as can be judged from the available data of these experiments, some factors seem to have been overlooked by Kolpatcka which would affect the interpretation of the results. Among these is the "lag" in the excretion of the products of metabolism, which would have an important influence upon the changes in the urine following a change in the ration, or during the first days of fasting. Moreover, the lag may be different for the three elements under discussion, and these differences may be influenced by the nature of the diet. The amounts of nitrogen, sulphur, and phosphorus in the feces are recorded but do not seem to have been included in determining the ratios. These quantities were generally not large, but in several cases the phosphorus of the feces

was over 10 per cent of the total excretion. The phosphoric acid determinations were in all cases made by titration with uranium acetate. For urine this may be considered fairly satisfactory; with meat and other materials containing appreciable amounts of iron the errors might be larger. Thus this investigation, while of great value and certainly rich in interest and suggestion, seems to neglect some factors which are still in need of investigation and which may appreciably affect the interpretation of the results obtained. These factors would be of even greater importance in experiments upon the human subject, where either the balance of income and outgo or the "lag," or both, may be more or less influenced by the mental and nervous condition, and where often a much larger proportion of the phosphorus leaves the body through the feces. If this fecal phosphate is an indigestible residue it should be deducted from the amount in the food before calculating the ratios discussed above. If, on the other hand, it has been metabolized and excreted into the intestine, it should be added to the amount eliminated in the urine. Much work is being done in the attempt to distinguish between the nitrogen of undigested residues and that of metabolic products. In the case of phosphorus this distinction is of greater relative importance, because the percentage of the ingested phosphorus eliminated in the feces is apt to be much larger than that of the ingested nitrogen.

PURPOSE AND PLAN OF THE EXPERIMENTS.

The work here reported comprises 10 experiments with man on a milk and bread diet, in each of which the digestibility of the nutrients of the food and the income and outgo of nitrogen, sulphur, and phosphorus were determined. In most cases two or more experiments were arranged in series, so that the change in diet gave an opportunity to determine whether the alteration in the excretion occurred simultaneously for the three elements studied; in other words, to compare the "lag" of sulphur and of phosphorus with that of nitrogen. The general purpose of the study was thus twofold—to accumulate additional determinations of the digestibility of bread and milk diet, and by collecting data regarding the comparative metabolism of nitrogen, sulphur, and phosphorus to prepare the way for the study of the latter elements in connection with certain of the nutrition investigations carried on by the Department of Agriculture. Attention has therefore been mainly directed to points which of themselves might not be of much interest, but which are likely to influence the methods of experimenting or the interpretation of the results. Among these points may be mentioned the question of "lag" already referred to, the influence of a change of routine, such as marked loss of sleep, the gain or loss of sulphur and phosphorus while the body is gaining or losing nitrogen, the proportion of urinary sulphur in forms other than sulphates and of phosphorus in forms other than phosphates, and the

question whether the large proportion of phosphorus which leaves the body by the feces is incapable of absorption, or is not absorbed because not needed, or has been metabolized in the body and excreted through the intestine.

For convenience of reference the digestibilities of the nutrients in the different experiments are first reported in the form which has been followed in previous bulletins of this series, after which the metabolism of nitrogen, sulphur, and phosphorus is discussed in a separate section.

ANALYTICAL METHODS.

All food materials used in the investigation were sampled at the time of use and all the feces were collected, dried, and analyzed. The methods of analysis were mainly those of the Association of Official Agricultural Chemists.[a] The determination of ether extract in the feces gave in some cases variable results and is not considered entirely satisfactory. The bread (soda crackers) and butter used in the experiments were generally prepared in advance in sufficient quantity for several experiments, thus reducing the number of analyses required. The milk used was obtained by mixing the entire product of a small local herd and was delivered in bottles. Previous experience had shown that the milk obtained from this source was almost uniform from day to day. In the present experiments a composite sample was prepared for each experimental period by taking a proportionate amount of the milk at the time of weighing the portion for each meal. In the fresh sample for each period the nitrogen and either the fat or the total solids was determined, after which a portion was dried, ground, and submitted to complete analysis. In case the partial analyses of samples of milk used in successive experiments of a continuous series showed no greater differences than occur in duplicate determinations on a single sample, the dried residues were ground together into a single composite sample for complete analysis.

Heat of combustion was determined by means of the Atwater-Blakeslee bomb calorimeter, as described in previous publications,[b] urine being previously dried on blocks of cellulose in the usual manner.[c]

Sulphur in foods and feces was oxidized to sulphuric acid, sometimes by fusion with sodium hydroxid and potassium nitrate in the usual manner and sometimes by burning the material in the bomb calorimeter. The latter method is quicker and more convenient in every way and, so far as we have employed it, gives the same results as the alkaline fusion method. The method of oxidation in the bomb

[a] U. S. Dept. Agr., Division of Chemistry Bul. 46.

[b] U. S. Dept. Agr., Office of Experiment Stations Bul. 21, p. 120; Connecticut Storrs Sta. Rpt. 1897, p. 199.

[c] Landw. Vers. Stat., 47 (1896), p. 297; U. S. Dept. Agr., Office of Experiment Stations Bul. 69, p. 23.

as here used was practically an adaptation of that given by Hempel.[a] The substance was pressed into a pellet and burned in the same manner as in determining the heat of combustion; then by means of a special coupling the gas in the bomb was allowed to escape slowly through an outlet tube having very narrow bore and was passed through bromin water in a U-tube containing glass beads. The moisture condensed on the cover and lining of the bomb was thoroughly washed out and united with the bromin water and rinsings from the U-tube, more bromin water added, if necessary, and the solution boiled to insure the oxidation to sulphuric acid of any sulphurous acid which may have been formed. The platinum capsule in which the pellet was burned was placed in a small beaker and heated with hydrochloric acid, to dissolve any sulphates in the ash.[b] This solution was then added to the one just mentioned and the whole filtered and precipitated with barium chlorid in the usual manner. As the solution always contained some iron from the igniting wire and the precipitates of barium sulphate were small and formed slowly, the latter were allowed to stand overnight in the cold before filtering. In the work here reported this method was used only to check some of the results obtained by the fusion method, but it has since been studied in some detail (see below).

In oxidizing the sulphur of foods and feces by the usual fusion method, from 1 to 3 grams of sample was melted with 7 to 12 grams of sodium hydroxid containing a little potassium nitrate; afterwards more nitrate was added in small portions until the oxidation was complete. The fusions were made in silver vessels heated by alcohol lamps. The fused mass after cooling was dissolved in water and twice evaporated to dryness with excess of hydrochloric acid, after which it was taken up with acidulated water and precipitated in the usual way. In estimating the total sulphur in urine, 40 cubic centimeters were evaporated to dryness in a silver dish or crucible and the residue treated as just described. The amount of sulphur introduced by the reagents used was determined and the corresponding corrections applied to the results obtained.

For the determination of phosphorus the material was oxidized either by means of caustic soda and potassium nitrate in the same manner as for the determination of sulphur, or by fusion with sodium carbonate and potassium nitrate in a similar manner. In the latter case the fusion was made in a platinum dish over a Bunsen burner. In either case the fused mass after cooling was dissolved in water, treated with

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[a] Ber. Deut. Chem. Gesell., 30 (1897), p. 202.

[b] In the presence of barium sulphate it would, of course, be necessary to fuse this residue. In the ordinary foods it seems safe to assume the absence of appreciable quantities of barium.

nitric acid in considerable excess, and the solution boiled down to small bulk, after which it was diluted, filtered if necessary, and the phosphoric acid determined by the molybdate-magnesia method, following the details adopted by the Association of Official Agricultural Chemists and using special care to insure the purity of the final precipitate.

The study of methods for the determination of sulphur and phosphorus has been continued since the completion of the experiments described in the bulletin. A comparison of the method of fusion with alkali and an oxidizing agent with that of combustion in oxygen showed practically identical results in the determination of sulphur and confirmed our preference for the latter method.

In the determination of phosphorus practically the same results were obtained whether the material was oxidized by means of carbonate and nitrate as above described, by combustion in oxygen as in the determination of sulphur, or by boiling with sulphuric acid and ammonium nitrate.

The details of this study of methods have been published elsewhere.[a]

COMPOSITION OF FOOD MATERIALS.

The milk used has already been described. For bread the material selected was commercial "soda crackers" or "soda biscuit," these being readily obtainable of practically uniform composition and easily kept without undergoing change or becoming distasteful. The butter was an ordinary product of good quality. The analyses of the food materials are given in Table 1.

TABLE 1.—*Composition of food materials.*

Laboratory number of sample.	Material.	Water.	Nitrogen.	Protein (N × 6.25).	Fat.	Carbohydrates.	Ash.	Energy per gram.	Sulphur.	Phosphorus.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Cals.*	*Per ct.*	*Per ct.*
650	Crackers (experiments Nos. 1–5)	9.32	1.610	10.06	6.21	72.32	2.09	4.221	0.130	0.110
651	Milk (experiments Nos. 1–3)	86.51	.535	3.34	4.42	4.98	.75	.767	.036	.096
652	Milk (experiments Nos. 4–5)	86.74	.542	3.39	4.26	4.86	.75	.778	.042	.096
653	Butter (experiments Nos. 1–4)	9.11	.180	1.13	86.97	--------	2.79	8.010	--------	--------
654	Milk (experiment No. 6)	87.08	.495	3.09	4.26	4.90	.67	.780	.034	.095
655	Crackers (experiment No. 6)	10.21	1.820	11.38	6.18	70.39	1.84	4.172	.143	.115
656	Crackers (experiments Nos. 7–10)	7.31	1.680	10.50	6.49	73.38	2.32	4.301	.130	.109
657	Milk (experiments Nos. 7–9)	86.73	.506	3.16	4.57	4.81	.73	.795	.034	.094
658	Milk (experiment No. 10)	86.50	.522	3.26	4.53	5.01	.70	.780	.032	.098

[a] Jour. Amer. Chem. Soc., **24** (1902), p. 1100.

COMPOSITION OF FECES.

As stated above, the feces were analyzed by the same methods as the food materials. The composition of the feces from the various experiments here reported is shown in Table 2, the results being given on the water-free basis, since the amount of water in the fresh feces has no bearing on the questions here studied.

TABLE 2.—*Composition of feces.*

Laboratory number of sample.	Feces.	Days.	Total amount. Partially dried.	Total amount. Water-free.	Dry matter in partially dried.	Nitrogen.	Protein (N×6.25).	Fat (ether extract).	Carbohydrates.	Ash.	Energy per gram.	Sulphur.	Phosphorus.
			Gms.	*Gms.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Calories.*	*Per cent.*	*Per cent.*
660	Experiment No. 1	4	97.5	95.1	97.51	3.12	19.50	13.63	38.28	28.59	5.686	0.268	3.26
661	Experiment No. 2	4	102.1	99.1	97.09	2.72	17.00	16.69	36.70	29.61	5.696	.246	2.96
662	Experiment No. 3	4	99.4	95.0	95.57	2.93	18.31	16.01	35.92	29.76	5.466	.272	3.26
663	Experiment No. 4	3	73.5	70.4	95.74	4.19	26.19	21.37	30.46	21.98	6.043	.385	1.86
664	Experiment No. 5	4	128.0	123.5	96.48	2.82	17.63	9.78	38.91	33.68	5.145	.270	3.56
665	Experiment No. 6	4	39.0	37.2	95.29	2.84	17.75	10.75	40.08	31.42	5.514	.246	3.97
666	Experiment No. 7	5	80.0	77.0	96.32	2.94	18.38	12.24	39.67	29.71	5.574	.253	3.88
667	Experiment No. 8	5	200.0	188.6	94.29	2.75	17.18	16.94	36.36	29.52	5.782	.229	3.77
668	Experiment No. 9	5	82.1	77.7	94.64	2.77	17.31	11.59	40.02	31.08	5.503	.238	3.22
669	Experiment No. 10	3	104.2	100.3	96.25	3.14	19.62	10.48	37.79	32.11	5.271	.295	4.11

EXPERIMENTS ON THE DIGESTIBILITY OF BREAD AND MILK.

GENERAL DESCRIPTION OF EXPERIMENTS.

The experiments here reported were made during the years 1900 and 1901. The subject was a healthy young man (the writer) with good appetite and apparently normal digestion and nutrition. The meals were taken in the laboratory, sometimes in the company of other young men engaged in similar experiments and sometimes alone. In each case (with a single unimportant exception, noted below) the diet was decided upon in advance, and was maintained uniform throughout the experiment or series of experiments. Exactly one-third of the day's ration was taken at each meal, and the meals were taken at nearly uniform hours: In the series of 1900, at 6.30 a. m., 12.30 p. m., and 6.30 p. m.; in that of 1901, at 7.30 a. m., 1 p. m. and 6.30 p. m. Excepting the butter, which was prepared in advance in weighed portions, the food required for each meal was weighed by the subject when used.

During the time covered by the experiments the subject was engaged partly in the analytical and other laboratory work connected with the investigation and partly in preparing for publication the results of previous studies. Little exercise was taken aside from that involved in the laboratory work, which was somewhat exacting.

Several of the experiments were arranged in series, and followed each other without intermission. In other cases the food taken on the day preceding the beginning of the experiment was practically the same as during the experimental period. Each experimental period began with breakfast, and the lampblack used to facilitate the separation of the feces was taken with this meal instead of with the preceding supper. In our experience it is very much easier to determine the point which marks the first appearance of the feces from a meal with which lampblack was taken than to decide exactly where the feces from such a meal end; apparently because, as would be expected, enough lampblack may sometimes adhere to the walls of the intestines to give more or less color to the feces from meals subsequent to that with which it was taken. It seems, therefore, decidedly preferable to take the lampblack with the first meal of the period and the first meal following the period, so that the point of separation shall be in each case the point at which the lampblack first appears in the feces. In each of the digestion experiments the urine was collected, beginning with the time at which the first breakfast was taken, and the nitrogen, sulphur, phosphorus, and heat of combustion determined.

The details of the digestion experiments are included in the following tables. These show the kind and amount of food eaten by the subject and the weight of the subject at the beginning and end of the experiment. The amount of protein, fat, and carbohydrates in each food material and in the feces was computed from the weight of each material multiplied by its percentage composition as shown in Tables 1 and 2. The heats of combustion, shown in the last column of the tables, were determined by burning the material in the bomb calorimeter and multiplying the total weight of food or feces by the heat of combustion of 1 gram, as thus determined. The differences between the total nutrients in the food eaten and those rejected in the feces are taken as a measure of the total amounts digested, although of course the feces do not consist entirely of undigested residues, but contain a relatively large amount of metabolic products.[a] The amounts of nutrients rejected in the feces, while not strictly representing the undigested portion of the food, do represent approximately the amounts which are not available to the body. The total amount of any particular kind of nutrient digested or available divided by the total amount of this nutrient in the food gives the percentage which is digestible or actually available to the body. These percentage values are called coefficients of digestibility or availability.

While the coefficients of digestibility of the different nutrients represent the proportion which the body actually utilizes, the corresponding value for the heat combustion of the food does not represent the

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[a] See discussion of this subject in Connecticut Storrs Sta. Rpts. 1896, p. 166, and 1897, p. 156.

actual amount of energy which the body obtains from the food absorbed from the alimentary canal. When protein is burned in the bomb calorimeter, the carbon is oxidized to carbon dioxid and the hydrogen to water, the nitrogen being reduced to the free state. When protein is burned in the body, however, the oxidation is not so complete. The nitrogen is excreted in the form of urea, uric acid, and other compounds, which also contain small amounts of carbon and hydrogen, together with some oxygen. In estimating the actual fuel values of the digestible nutrients of the food, allowance must be made for these incompletely oxidized residual products which are excreted by the kidneys. Urea is the most abundant of these excretory products, and it has frequently been assumed that all of the nitrogen excreted in the urine is thus combined, and allowance is made for the heat of combustion of the amount of urea corresponding to the amount of nitrogen found in the urine. According to this last supposition, 0.87 calorie of the energy latent in each gram of digestible protein would be lost to the body in the urea formed from the nitrogen of the protein.[a] In a considerable number of actual determinations of the ratio of the nitrogen to heat of combustion in urine of healthy men made by Atwater and associates at Middletown, Conn., the average heat of combustion of the organic matter in the urine corresponding to 1 gram of digestible protein amounts to 1.25 calories. In the experiments here reported the actual heat of combustion was determined in each instance. The average of these determinations corresponded to 1.20 calories per gram of digestible protein.

The results of the individual tests are given below. Following the tabular statement of the details of each experiment is a paragraph showing the nitrogen balance; that is, whether the subject gained or lost nitrogen during the test. The discussion of the nitrogen balance, as well as that of sulphur and phosphorus, will be found in another section of this report (pp. 31–46).

DIGESTION EXPERIMENT NO. 1.

This experiment began with breakfast July 20, 1900, and continued four days. The weight of the subject (without clothing) at the beginning was 60.1 kilograms, at the end 60 kilograms.

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[a] Urea contains 46.67 per cent nitrogen and has a heat of combustion of 2.54 calories per gram. One gram of protein (16 per cent nitrogen) would yield ($16 \div 46.67 =$) 0.342 gram urea with a heat of combustion of ($2.54 \times 0.342 =$) 0.87. See also U. S. Dept. Agr., Office of Experiment Stations Bul. 53, pp. 27 and 28.

TABLE 3.—*Results of digestion experiment No. 1 (serial No. 324).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N×6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
650	Crackers	1,200	1,063.0	120.7	74.5	867.8	25.1	19.32	5,065
651	Milk	8,160	1,039.5	272.5	360.6	406.4	61.2	43.66	6,258
653	Butter	160	140.9	1.8	139.1		4.5	.29	1,282
	Total		2,243.4	395	574.2	1,274.2	90.8	63.27	12,605
660	Feces (water free)	95.1	67.9	18.5	13	36.4	27.2	2.97	541
	Amount digested		2,175.5	376.5	561.2	1,237.8	63.6	60.30	12,064
	Per cent digested		97.0	95.3	97.7	97.2	70	95.3	95.7
	Nitrogen and heat of combustion of urine							57.23	434
	Energy of food oxidized in the body								11,630
	Per cent of energy utilized								92.3

During this experiment the subject eliminated 2,550 grams of urine, containing 57.23 grams of nitrogen. The average balance per day was therefore: Income in food, 15.82 grams; outgo in urine, 14.31 grams, and in feces, 0.74 gram; implying a gain to the body of 0.77 gram of nitrogen, corresponding to 4.81 grams of protein.

DIGESTION EXPERIMENT NO. 2.

This experiment began with breakfast July 24, 1900, and continued four days. The weight of the subject (without clothing) at the beginning was 60 kilograms, at the end 61 kilograms.

TABLE 4.—*Results of digestion experiment No. 2 (serial No. 325).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N×6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
650	Crackers	1,200	1,063.0	120.7	74.5	867.8	25.1	19.32	5,065
651	Milk	8,160	1,039.5	272.5	360.6	406.4	61.2	43.66	6,258
653	Butter	160	140.9	1.8	139.1		4.5	.29	1,282
	Total		2,243.4	395	574.2	1,274.2	90.8	63.27	12,605
661	Feces (water free)	99.1	69.7	16.8	16.5	36.4	29.3	2.70	564
	Amount digested		2,173.7	378.2	557.7	1,237.8	61.5	60.57	12,041
	Per cent digested		96.9	95.8	97.1	97.2	67.7	95.8	95.5
	Nitrogen and heat of combustion of urine							58.69	438
	Energy of food oxidized in the body								11,603
	Per cent of energy utilized								92.3

During this experiment the subject eliminated 3,451 grams of urine, containing 58.69 grams of nitrogen. This makes the average nitrogen balance per day as follows: Income in food, 15.82 grams; outgo in urine, 14.67 grams, and in feces, 0.67 gram; indicating a gain to the body of 0.48 gram of nitrogen, corresponding to 3 grams of protein.

DIGESTION EXPERIMENT NO. 3.

This experiment began with breakfast July 28, 1900, and continued four days. The weight of the subject (without clothing) at the beginning was 61 kilograms, at the end 60.9 kilograms.

TABLE 5.—*Results of digestion experiment No. 3 (serial No. 326).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N × 6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
650	Crackers	1.200	1,063.0	120.7	74.5	867.8	25.1	19.32	5.065
651	Milk	8,160	1,039.5	272.5	360.6	406.4	61.2	43.66	6,258
653	Butter	160	140.9	1.8	139.1		4.5	.29	1.282
	Total		2,243.4	395	574.2	1,274.2	90.8	63.27	12,605
662	Feces (water free)	95	66.7	17.4	15.2	34.1	28.3	2.78	519
	Amount digested		2,176.7	377.6	559	1,240.1	62.5	60.49	12,086
	Per cent digested		97	95.6	97.4	97.3	68.8	95.60	95.9
	Nitrogen and heat of combustion of urine							56.65	435
	Energy of food oxidized in the body								11.651
	Per cent of energy utilized								92.4

During this experiment the subject eliminated 4,071 grams of urine, containing 56.65 grams of nitrogen. This makes the average nitrogen balance per day as follows: Income of food, 15.82 grams; outgo in urine, 14.16 grams, and in feces, 0.70 gram, indicating a gain of 0.96 gram of nitrogen or 6 grams of protein.

DIGESTION EXPERIMENT NO. 4.

This experiment began with breakfast August 11, 1900, and continued four days. The weight of the subject (without clothing) at the beginning was 60.6 kilograms, at the end 62 kilograms.

TABLE 6.—*Results of digestion experiment No. 4 (serial No. 327).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N×6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
650	Crackers	1,620	1,435.1	163.0	100.6	1,171.5	33.9	26.08	6,838
652	Milk	4,000	500.4	135.6	170.4	194.4	30	21.68	3,112
653	Butter	240	211.4	2.7	208.7		6.7	.43	1,922
	Total		[a]1,610.2	226	359.8	1,024.4	53	36.14	8,904
			2,146.9	301.3	479.7	1,365.9	70.6	48.19	11,872
663	Feces (water free) [a]	70.4	41.2	13.8	11.3	16.1	11.6	2.21	319
	Amount digested		1,569	212.2	348.5	1,008.3	41.4	33.93	8,585
	Per cent digested		97.4	93.9	96.9	98.4	78.1	93.9	96.4
	Nitrogen and heat of combustion of urine							31.28	258
	Energy of food oxidized in the body								8,327
	Per cent of energy utilized								93.5

[a] Three-fourths of total amount; urine for first day lost.

The urine for the first day of this experiment was lost. During the remaining three days the subject eliminated 1,990 grams of urine, containing 31.28 grams of nitrogen. This makes the average nitrogen balance per day as follows: Income in food, 12.05 grams; outgo in urine, 10.43 grams, and in feces, 0.74 gram; indicating that the body gained 0.88 gram of nitrogen, or 5.50 grams of protein per day.

DIGESTION EXPERIMENT NO. 5.

This experiment began with breakfast August 15, 1900, and continued four days. The weight of the subject (without clothing) at the beginning was 62 kilograms, at the end 60.8 kilograms.

TABLE 7.—*Results of digestion experiment No. 5 (serial No. 328).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N×6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
650	Crackers	480	425.2	48.3	29.8	347.1	10	7.73	2,026
652	Milk	12,240	1,531.2	415	521.4	594.8	91.8	66.34	9,523
	Total		1,956.4	463.3	551.2	941.9	101.8	74.07	11,549
664	Feces (water free)	123.5	81.9	21.8	12.1	48	41.6	3.48	635
	Amount digested		1,874.5	441.5	539.1	893.9	60.2	70.59	10,914
	Per cent digested		95.8	95.3	97.8	94.9	59.1	95.3	94.5
	Nitrogen and heat of combustion of urine							66.01	487
	Energy of food oxidized in the body								10,427
	Per cent of energy utilized								90.3

During this experiment the subject eliminated 7,889 grams of urine, containing 66.01 grams of nitrogen, making the average nitrogen balance per day as follows: Income in food, 18.52 grams; outgo in urine, 16.50 grams, and in feces, 0.87 gram; implying a gain of 1.15 grams of nitrogen or 7.19 grams of protein.

DIGESTION EXPERIMENT NO. 6.

This experiment began with breakfast July 4, 1901, and continued four days. The weight of the subject (without clothing) at the beginning was 61.45 kilograms, at the end 60.13 kilograms.

TABLE 8.—*Results of digestion experiment No. 6 (serial No. 329).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N×6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
655	Crackers	480	422.2	54.6	29.7	337.9	8.8	8.74	2,003
654	Milk	6,120	749.6	189.1	260.7	299.8	41	30.29	4,774
	Total		1,171.8	243.7	290.4	637.7	49.8	39.03	6,777
665	Feces (water free)	37.2	25.5	6.6	4	14.9	11.7	1.06	205
	Amount digested		1,146.3	237.1	286.4	622.8	38.1	37.97	6,572
	Per cent digested		97.8	97.3	98.6	97.7	76.5	97.3	97
	Nitrogen and heat of combustion of urine							43.77	350
	Energy of food oxidized in the body								6,222
	Per cent of energy utilized								91.8

During this experiment the subject eliminated 3,232 grams of urine, containing 43.77 grams of nitrogen. The average nitrogen balance per day was therefore: Income in food, 9.76 grams; outgo in urine, 10.94 grams, and in feces, 0.27 gram; indicating a loss of 1.45 grams of nitrogen or 9.06 grams of protein.

DIGESTION EXPERIMENT NO. 7.

This experiment began with breakfast July 14, 1901, and continued five days. The weight of the subject (without clothing) at the beginning was 60 kilograms, at the end 59.2 kilograms.

TABLE 9.—*Results of digestion experiment No. 7 (serial No. 330).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N × 6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
656	Crackers	750	677.8	78.8	48.7	550.3	17.4	12.60	3,226
657	Milk	7,500	940.4	237	342.7	360.7	54.7	37.95	5,963
	Total		1,618.2	315.8	391.4	911	72.1	50.55	9.189
666	Feces (water free)	77	54.1	14.2	9.4	30.5	22.9	2.26	429
	Amount digested		1,564.1	301.6	382	880.5	49.2	48.29	8,760
	Per cent digested		96.7	95.5	97.6	96.7	68.2	95.5	95.3
	Nitrogen and heat of combustion of urine							57.53	460
	Energy of food oxidized in the body								8,300
	Per cent of energy utilized								90.3

During this experiment the subject eliminated 3,027 grams of urine, containing 57.53 grams of nitrogen. This makes the average daily nitrogen balance as follows: Income in food, 10.11 grams; outgo in urine, 11.51 grams, and in feces, 0.45 gram; corresponding to a daily loss of 1.85 grams of nitrogen or 11.56 grams of protein.

DIGESTION EXPERIMENT NO. 8.

This experiment began with breakfast July 19, 1901, and continued five days. The weight of the subject (without clothing) at the beginning was 59.2 kilograms, at the end 60.7 kilograms.

TABLE 10.—*Results of digestion experiment No. 8 (serial No. 331).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N × 6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
656	Crackers	1,500	1,355.5	157.5	97.3	1,100.7	34.8	25.20	6,452
657	Milk	15,000	1,881	474	685.5	721.5	109.5	75.90	11,925
	Total		3,236.5	631.5	782.8	1,822.2	144.3	101.10	18,377
667	Feces (water free)	188.6	132.9	32.4	31.9	68.6	55.7	5.19	1,090
	Amount digested		3,103.6	599.1	750.9	1,753.6	88.6	95.91	17,287
	Per cent digested		95.9	94.9	95.9	96.2	61.4	94.9	94.1
	Nitrogen and heat of combustion of urine							77.62	606
	Energy of food oxidized in the body								16,681
	Per cent of energy utilized								90.8

During this experiment, which followed No. 7 without intermission, the subject eliminated 5,223 grams of urine containing 77.62 grams of nitrogen. This makes the average nitrogen balance per day as follows: Income in food, 20.22 grams; outgo in urine, 15.52 grams, and in feces, 1.04 grams; implying a storage in the body of 3.66 grams of nitrogen, corresponding to 22.87 grams of protein.

DIGESTION EXPERIMENT NO. 9.

This experiment began with breakfast July 24, 1901, and continued five days. The weight of the subject (without clothing) at the beginning was 60.7 kilograms, at the end 59.3 kilograms.

TABLE 11.—*Results of digestion experiment No. 9 (serial No. 332).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N×6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
656	Crackers	750	677.8	78.8	48.7	550.3	17.4	12.60	3,226
657	Milk	7,500	940.4	237.0	342.7	360.7	54.7	37.95	5,963
	Total		1,618.2	315.8	391.4	911.0	72.1	50.55	9,189
668	Feces (water free)	77.7	53.6	13.5	9.0	31.1	24.1	2.15	428
	Amount digested		1,564.6	302.3	382.4	879.9	48.0	48.40	8,761
	Per cent digested		96.7	95.7	97.7	96.6	66.6	95.7	95.3
	Nitrogen and heat of combustion of urine							64.33	479
	Energy of food oxidized in the body								8,282
	Per cent of energy utilized								90.1

During this experiment, which followed No. 8 without intermission and which was a duplicate of No. 7, the subject eliminated 4,310 grams of urine containing 64.33 grams of nitrogen. The average daily nitrogen balance was therefore: Income in food, 10.11 grams; outgo in urine, 12.87 grams, and in feces, 0.43 gram; indicating a loss of 3.19 grams of nitrogen, or 19.94 grams of protein.

DIGESTION EXPERIMENT NO. 10.

This experiment began with breakfast July 29, 1901, and continued three days. The weight of the subject (without clothing) at the beginning was 59.3 kilograms, at the end 60 kilograms.

TABLE 12.—*Results of digestion experiment No. 10 (serial No. 333).*

Laboratory number of sample.	Kind of food.	Weight of material.	Total organic matter.	Protein (N × 6.25).	Fat.	Carbohydrates.	Ash.	Nitrogen.	Heat of combustion (determined).
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Calories.*
656	Crackers	900	813.3	94.5	58.4	660.4	20.9	15.12	3,871
658	Milk	9,000	1,152.0	293.4	407.7	450.9	63.0	46.98	7,020
	Total		1,965.3	387.9	466.1	1,111.3	83.9	62.10	10,891
669	Feces (water free)	100.3	68.1	19.7	10.5	37.9	32.2	3.15	529
	Amount digested		1,897.2	368.2	455.6	1,073.4	51.7	58.95	10,362
	Per cent digested		96.5	94.9	97.7	96.6	61.6	94.9	95.2
	Nitrogen and heat of combustion of urine							49.74	374
	Energy of food oxidized in the body								9,988
	Per cent of energy utilized								91.7

This experiment followed No. 9 without intermission. The diet was nearly the same as in No. 8. During the three days of this experiment the subject eliminated 4,290 grams of urine containing 49.74 grams of nitrogen. The average nitrogen balance per day was therefore: Income in food, 20.70 grams; outgo in urine, 16.58 grams, and in feces, 1.05 grams; indicating a gain of 3.07 grams of nitrogen, corresponding to 19.19 grams of protein.

RESULTS OF DIGESTION EXPERIMENTS.

In Table 13 are summarized the results obtained in the various experiments on the digestibility of the total food eaten. Although the diet was composed in each case of bread (in the form of soda crackers) and milk, with butter in some cases, the relative proportions of these two food materials, as well as the quantities taken, varied in the different experiments, as will be seen from the details of the experiments given above.

TABLE 13.—*Coefficients of digestibility of nutrients and availability of energy.*

Experiment number.	Kind of food.	Protein.	Fat.	Carbohydrates.	Ash.	Energy.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	Bread, butter, and milk	95.3	97.7	97.2	70.0	92.3
2	do	95.8	97.1	97.2	67.7	92.3
3	do	95.6	97.4	97.3	68.8	92.4
4	do	93.9	96.9	98.4	78.1	93.5
5	Bread and milk	95.3	97.8	94.9	59.1	90.3
6	do	97.3	98.6	97.7	76.5	91.8
7	do	95.5	97.6	96.7	68.2	90.3
8	do	94.9	95.9	96.2	61.4	90.8
9	do	95.7	97.7	96.6	66.6	90.1
10	do	94.9	97.7	96.6	61.6	91.7

As explained above, the experiments were varied in order to study the digestibility under different circumstances, so that an average of the results obtained would have little value except as these variations are taken into consideration. It will be noted that the results of experiment No. 6 differ markedly from all the others in the larger percentage of the protein digested, the digestibility of the fat being also increased but not to such a marked degree. This result is not due to the relative proportions of bread and milk in the diet, since in this respect the experiment is intermediate between experiments Nos. 5 and 7. The amount of protein taken in the food was somewhat less than in experiment No. 7, and very much less than in experiment No. 5. This fact would of course be favorable to the more complete absorption of the protein, as would also the circumstance that during the week previous to the test the subject had eaten less food than usual. These circumstances may account for the rather unusual figures obtained in this period, and as the feces were collected, dried, and weighed by the subject himself it would seem improbable that any serious loss could have occurred without being detected. Nevertheless, the amounts of total dry matter, nitrogen, and phosphorus found in the feces for this period are so small that the results are given with some hesitation, and in comparing the determined and calculated figures for digestibility we have averaged the experiments both with and without No. 6.

Table 14 shows for each experiment and for the average of all the experiments: (1) The percentage of protein actually digested, as determined; (2) the digestibility as calculated, assuming that 85 per cent of the protein from cereals and 97 per cent of the protein from milk were digested, and (3) the figures calculated on the assumption that 90 per cent of the protein of the bread and 97 per cent of the protein of the milk were digested.

TABLE 14.—*Coefficients of digestibility of protein, calculated and determined.*

	Results actually found.	Results calculated, assuming that 85 per cent of bread protein and 97 per cent of milk protein were digested.	Results calculated, assuming that 90 per cent of bread protein and 97 per cent of milk protein were digested.
	Per cent.	*Per cent.*	*Per cent.*
Experiment No. 1	95.3	93.3	94.9
Experiment No. 2	95.8	93.3	94.9
Experiment No. 3	95.6	93.3	94.9
Experiment No. 4	93.9	90.5	93.2
Experiment No. 5	95.3	95.8	96.3
Experiment No. 6	97.3	93.9	95.3
Experiment No. 7	95.5	94.0	95.3
Experiment No. 8	94.9	94.0	95.3
Experiment No. 9	95.7	94.0	95.3
Experiment No. 10	94.9	94.0	95.3
Average of all	95.4	93.6	95.1
Average, omitting experiment No. 6	95.2	93.6	95.0

It will be seen that the digestibility of the protein of the diet, as calculated on the assumption that 85 per cent represents the digestibility of bread protein and 97 per cent that of milk protein are in 9 out of the 10 cases noticeably lower than the results actually obtained, the average being 1.8 or 1.6 per cent lower than the average actual value, according as we do or do not include experiment No. 6.

If, however, we assume that 90 per cent of the bread protein was digestible, and use the same factor as before (97 per cent) for the milk, we find that (with the exception of experiment No. 6) the calculated and determined values agree in every instance within 1 per cent, while the averages agree within one-quarter of 1 per cent, a variation which may well be considered as negligible.

The factor 90 per cent for the digestibility of the bread protein was suggested by the fact that this is about the value found for white bread by Woods and Merrill[a] in an extended series of experiments with a number of different subjects, and also in tests with one of the four subjects employed by Snyder.[b]

As might be expected from the fact that fat is supplied in an emulsified and readily available form in milk, its digestibility in these experiments was rather higher than is usually found. A detailed comparison, such as that given for the protein, is, however, impracticable, (1) because of the impossibility of distinguishing between animal and vegetable fats in the crackers used, and (2) because those portions of the feces designated "fats" and "carbohydrates" really consist largely of other substances.

During experiment No. 2, in which the diet was the same as in experiments Nos. 1 and 3, there was (as will be more fully described beyond, p. 35) a very considerable loss of sleep. This, however, does not seem to have had any appreciable effect upon the proportion of either of the nutrients digested.

Experiments Nos. 7, 8, 9, and 10 throw some light upon the digestibility of liberal and restricted diets. These were carried out in series, and the relative proportions of milk and bread were uniform throughout. The amount eaten per day was, however, twice as great in experiments Nos. 8 and 10 as in experiments Nos. 7 and 9. On the smaller diet the percentage digested was slightly higher. The difference is quite small, less than 1 per cent, but as all other experimental conditions were carefully maintained uniform, and as the agreement between the similar experiments is almost complete, it would seem that the better digestibility shown by experiments Nos. 7 and 9 over experiments Nos. 8 and 10 must be attributed to the fact that less food was taken. Larger but more variable differences have already been observed by Snyder (loc. cit.) in similar experiments.

Experiments Nos. 7 to 10 were carried out without intermission, and

[a] U. S. Dept. Agr., Office of Experiment Stations Bul. 85, p. 32.
[b] U. S. Dept. Agr., Office of Experiment Stations Bul. 101, p. 33.

covered a period of eighteen days. Experiments Nos. 1 to 3 covered similarly a period of twelve days. The results obtained from these experiments make it evident that an extremely simple diet may be continued for a very considerable number of days without necessarily diminishing its digestibility.

COMPARISON OF THE METABOLISM OF NITROGEN, SULPHUR, AND PHOSPHORUS.

As has been explained, the experiments above discussed as digestion tests were also designed to include a study of the comparative metabolism of nitrogen, sulphur, and phosphorus. In each experiment the diet was uniform and the urine for each twenty-four hours was collected and examined. Aliquot portions of each day's urine were mixed to give a composite sample representing the entire period. Nitrogen was determined in the urine of each day, and the results were verified by the analysis of the composite sample.

Phosphates in the daily urines were determined volumetrically by titration with standardized uranium acetate solution in the usual manner. The total phosphorus of the urine for the whole period was determined as described in the section on analytical methods, above. It will be seen from the results as tabulated below that the sum of the figures obtained by titration of the daily urines ranges in the different experiments from 95.5 to 98 per cent of the total by the gravimetric method in the composite for the period. These variations are very likely due as much to errors in the volumetric determinations as to differences in the amount of "organic" phosphates present. If the methods and manipulation were free from error the results would indicate from 0.02 to 0.05 gram of phosphorus (or 0.04 to 0.12 gram P_2O_5) per day eliminated in forms not precipitated by uranium. This amount is so small that it appears quite sufficient to use the volumetric method when one desires merely to follow the general course of the phosphorus excretion, determining the total phosphorus by the standard gravimetric method in cases where an accurate balance of income and outgo is to be determined. In this connection it may be noted that recent investigations by Ceconi and others[a] of the so-called organic phosphates of the urine have given quite variable results and have not tended to emphasize the importance of the small amount of phosphorus thus combined.

On account of unavoidable interruptions it was impossible in the experiments carried out in 1900 to determine sulphur in the urine of each day. The amount of total sulphur and of sulphate sulphur was, however, determined for each period. The same determinations were

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[a] 7 Verhandl. Cong. Innere Med. Rome, 1896; abs. in Jahrb. Thier.-Chem., 27 (1897), p. 362. Jolly, Compt. Rend. Acad. Sci. Paris, 127 (1898), p. 118. Oertel, Ztschr. Physiol. Chem., 26 (1898), p. 123. Keller, Ibid., 29 (1900), p. 146.

included in experiment No. 6. In experiments Nos. 7 to 10 the sulphate sulphur was determined for each day and the total sulphur for each period. Comparing the "sulphate" and "total" sulphur in the different experiments, it would appear that from 83.1 to 89.6 per cent of the sulphur in the urine was in the form of sulphates. The sulphur in forms other than sulphates—so-called "neutral" sulphur—has recently been studied by Reale and Velardi,[a] Harnack and Kleine,[b] Freund,[c] Petry,[d] and doubtless others, and will probably repay further investigation. In the present experiments, however, time did not permit of any study of this question. Neither did the analyses include the separate determination of the ethereal sulphates which, as the protein consumed came principally from milk, were probably present in less than the usual proportions.[e]

The final results of the examinations of the urine are brought together in Table 15, which shows the data for each experimental day, as well as the total for each of the ten periods. Partial analyses of the urine for the four days immediately following experiment No. 5 are also given.

TABLE 15.—*Data of examination of urine in experiments Nos. 1–10.*

Experiment number.	Date.	Total amount voided.	Specific gravity.	Nitrogen.	Sulphur.		Phosphorus.		Heat of combustion.
					As sulphates.	Total.	By titration.	Total.	
	1900.	*Grams.*		*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Cals.*
1	July 20–21	817	1.0280	15.38			1.30		
	July 21–22	902	1.0270	13.89			1.34		
	July 22–23	993	1.0260	14.28			1.40		
	July 23–24	838	1.0280	13.68			1.41		
	Total	3,550		57.23	3.37	3.80	5.45	5.63	433.8
2	July 24–25	852	1.0280	13.96			1.28		
	July 25–26	782	1.0300	14.04			1.34		
	July 26–27	851	1.0310	15.63			1.43		
	July 27–28	966	1.0285	15.06			1.66		
	Total	3,451		58.69	3.46	3.86	5.71	5.90	438.2
3	July 28–29	1,160	1.0250	15.65			1.51		
	July 29–30	978	1.0270	13.67			1.34		
	July 30–31	952	1.0270	13.51			1.26		
	July 31–August 1	1,001	1.0255	13.82			1.40		
	Total	4,071		56.65	3.24	3.74	5.51	5.66	434.7
4	August 12–13	609	1.0325	10.19			.94		85.6
	August 13–14	711	1.0305	10.92			1.06		88.7
	August 14–15	670	1.0305	10.17			1.02		83.4
	Total	1,990		31.28	1.85	2.16	3.02	3.10	257.7
5	August 15–16	2,180	1.0130	15.12			1.48		110.6
	August 16–17	2,130	1.0120	16.07			1.72		117.4
	August 17–18	1,859	1.0145	16.98			1.79		127.3
	August 18–19	1,720	1.0150	17.84			1.78		131.8
	Total	7,889		66.01	3.94	4.48	6.77	6.96	487.1

a Studii di clinica medica, Napoli, 1895; abs. in Arch. Verdauungskrankh., 2 (1896–97), p. 141.

b Ztschr. Biol., 37 (1899), p. 417.

c Ztschr. Physiol. Chem., 29 (1900), p. 24.

d Ibid., 29 (1900), p. 45.

e See results by Laquer, Verhandl. Cong. Innere Med., 16 (1898), p. 546; abs. in Jahrb. Their.-Chem., 28 (1898), p. 336.

TABLE 15.—*Data of examination of urine in experiments Nos. 1–10*—Continued.

Experiment number.	Date.	Total amount voided.	Specific gravity.	Nitrogen.	Sulphur.		Phosphorus.		Heat of combustion.
					Assulphates.	Total.	By titration.	Total.	
	1900—Cont'd.	*Grams.*		*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Cals.*
	August 19–20	1,698	1.0150	18.12	....	....	1.76	....	....
	August 20–21	1,730	1.0150	18.72	....	....	1.70	....	....
	August 21–22	1,545	1.0170	18.08	....	....	1.71	....	....
	August 22–23	1,835	1.0150	17.78	....	....	1.69	....	....
	Total	6,808	....	72.82	4.27	....	6.86	....	....
	1901.								
6	July 4–5	879	1.0230	10.58	....	....	.96	....	....
	July 5–6	641	1.0275	10.42	....	....	1.01	....	....
	July 6–7	760	1.0270	11.32	....	....	1.10	....	....
	July 7–8	952	1.0215	11.45	....	....	1.21	....	....
	Total	3,232	....	43.77	2.57	3.00	4.28	4.46	350.3
7	July 14–15	681	1.0320	11.65	.68	....	.84	....	....
	July 15–16	605	1.0320	11.12	.67	....	.88	....	....
	July 16–17	620	1.0320	11.35	.72	....	.99	....	....
	July 17–18	566	1.0350	11.66	.68	....	1.04	....	....
	July 18–19	555	1.0350	11.75	.69	....	1.00	....	....
	Total	3,027	....	57.53	3.44	4.14	4.75	4.98	459.8
8	July 19–20	687	1.0340	14.83	.93	....	1.19	....	....
	July 20–21	874	1.0310	15.81	.90	....	1.43	....	....
	July 21–22	1,460	1.0200	15.19	.89	....	1.42	....	....
	July 22–23	1,110	1.0270	14.91	.96	....	1.60	....	....
	July 23–24	1,092	1.0290	16.88	1.04	....	1.57	....	....
	Total	5,223	....	77.62	4.72	5.28	7.21	7.44	605.9
9	July 24–25	790	1.0280	12.64	.77	....	1.25	....	....
	July 25–26	783	1.0290	12.57	.75	....	1.21	....	....
	July 26–27	936	1.0270	13.01	.79	....	1.37	....	....
	July 27–28	902	1.0240	12.73	.72	....	1.25	....	....
	July 28–29	899	1.0260	12.88	.78	....	1.21	....	....
	Total	4,310	....	64.33	3.81	4.39	6.29	6.59	478.8
10	July 29–30	1,340	1.0200	16.23	1.02	....	1.33	....	....
	July 30–31	1,750	1.0200	16.71	1.05	....	1.44	....	....
	July 31–August 1	1,200	1.0240	16.80	1.04	....	1.51	....	....
	Total	4,290	....	49.74	3.11	3.64	4.28	4.46	374.1

With the exception of experiment No. 6, the experiments fall into three series, as will be seen from the dates given in the table. The general occupation and habits of the subject were similar in all, except as modified for the experiment in which the effect of loss of sleep was studied, and have already been described. The first series included three four-day experiments (Nos. 1, 2, and 3) carried out in the latter part of July, 1900. The bread and milk diet was taken for a day and a half before the beginning of the first experiment. The diet was entirely uniform throughout, except that on the first day 60 grams and on the second day 20 grams of butter were taken, after which 40 grams per day were taken throughout. The fuel value of the diet was thus a little above the average on the first and a little below the average on the second day, but was the same for each of the three periods,

and except for this slight variation in the amount of fat taken on the first two days the daily diet was uniform throughout the twelve days covered by the series.

The second series, including experiments Nos. 4 and 5, was begun ten days after the conclusion of the first series. During most of the intervening time the subject had followed the usual routine and lived on a rather simple mixed diet consisting largely of bread, milk, and fruit. Three days shortly before the beginning of experiment No. 4 (August 7 to 9 inclusive) were, however, spent in another place and under somewhat different conditions, the work being more active and the diet more abundant and varied, and consisting more largely of meat. Experiment No. 4 was really begun on the morning of August 11, and the feces collected correspond to a period of four days. Unfortunately the urine of the first day was lost, so that as a metabolism experiment it covers only three days preceded by a day in which exactly the same diet was taken. In calculating the balance of income and outgo for the three days it is assumed that the elimination of feces was practically uniform. As compared with the preceding experiments the diet in experiment No. 4 was about normal as regards fuel value but low in protein. While this diet was evidently sufficient for the needs of the body, the large amount of bread and butter was not appetizing to the subject, who during this experiment felt a little less vigorous than usual, though perfectly well. After four days on this diet experiment No. 5 was begun, the diet being changed by omitting the butter, reducing the bread to less than one-third and greatly increasing the amount of milk, so that the diet had nearly the same fuel value as in experiment No. 4, but furnished over 50 per cent more protein.

In the third series (experiments Nos. 7 to 10) carried out in July, 1901, the diet was qualitatively uniform throughout the eighteen days covered; that is, the diet consisted of bread and milk in the same relative proportions. The amounts taken daily were, however, twice as great in experiments Nos. 8 and 10 as in experiments Nos. 7 and 9. The daily routine was similar to that followed in the preceding experiments, except that the experimental day was begun at 7.30 a. m., instead of 6.30 a. m.

The results obtained can be best discussed by considering separately the different points on which the experiments were designed to throw some light.

INFLUENCE OF LOSS OF SLEEP.

Roeske[a] as the result of an extended study of the course of the phosphorus excretion during the day concluded that the degree of

[a] Ueber den Verlauf der Phosphorsäure-Ausscheidung beim Menschen. Inaug. Diss., Greifswald, 1897.

mental and vital activity, and especially the alternation of sleeping and waking periods, had a greater influence upon the excretion of phosphorus than did the food ingested. Thus, when the urine was collected and the phosphorus determined for each two-hour period from the time of rising—6 a. m.—till that of retiring—11 p. m.—the curve representing the excretion was quite similar from day to day. This normal course of the excretion was not greatly altered by changing the diet or even by omitting a meal entirely, but was strikingly changed when the subject rose two hours before the usual time in the morning. The food and feces were not analyzed and apparently the diet was not the same on the different "normal" days, so that the conclusions were necessarily based more largely upon alterations in the form of the curve representing the excretion than upon the total amount excreted during the day, and as neither nitrogen nor sulphur was determined there is nothing to indicate whether or not the changes in phosphorus metabolism accompanied similar changes in the metabolism of proteid material. In view of these questions it seemed desirable in beginning the present investigation to give some attention to this matter, in order to ascertain whether unusual precautions as to regularity of hours would be necessary in experiments in which the metabolism of phosphorus was to be studied in comparison with that of nitrogen. This point was studied by greatly reducing the time spent in sleep in experiment No. 2, all of the other conditions (which have already been described) being the same as in experiments Nos. 1 and 3. Experiment No. 1 ended and experiment No. 2 began with breakfast of July 24, 1900. That night the subject slept but two and one-half hours, the following night four hours, and the third night no sleep was taken. The subject then returned to his usual routine, sleeping about seven hours each day. The waking hours of the first night were spent upon mental work of the kind to which the subject was accustomed. On the second and third nights, after the usual hour of retiring, the time was passed in reading light literature. Only on the second night was there difficulty in remaining awake. Throughout each day and until 10 or 11 o'clock in the evening the subject was engaged upon his usual duties and did not feel any distinct effect of the loss of sleep, except a slight nervousness on the day following the third night—that on which no sleep was taken.

The loss of sleep resulted in an increased elimination of each of the three elements studied, but as the sulphur was determined simply by periods, only the nitrogen and phosphorus can be compared in detail. It will be seen from Table 15 that the increased elimination does not appear until after the second night and then continues for two days after the return to the usual routine, thus running over into the following period (experiment No. 3). The results are best shown, therefore,

not by a comparison of the totals for the three periods, but by averaging the days in which the increase is found actually to have occurred and comparing with the preceding and following days. Thus we find for five "normal" days, beginning July 21, an average of 13.97 grams nitrogen and 1.355 grams of phosphorus, with the following ratio: N : P :: 100 : 9.66. The following three days, which show the effect of the loss of sleep, average 15.45 grams nitrogen and 1.538 grams phosphorus, with a ratio of 100 : 9.96; while the three days next following, when the elimination is again normal, average 13.67 grams nitrogen and 1.328 grams phosphorus, the ratio being 100 : 9.65.

In view of the amount of sleep lost the total increased elimination seems quite small both in the case of nitrogen and that of phosphorus, and it is evident that the latter was only slightly more affected than the former, since the change in the ratio is no larger than would often be found on comparing successive "normal" days when all the conditions appear to be uniform.

The relative fluctuations in the amounts of nitrogen and phosphorus eliminated daily during this series is shown graphically in fig. 1, in which the solid line represents the excretion of nitrogen and the dotted line that of phosphorus.

The elimination of sulphur as measured by the four-day periods ran very closely parallel with that of nitrogen throughout this series, the ratio N : S being in the first period as 100 : 6.64, in the second as 100 : 6.58, and in the third as 100 : 6.60.

LAG OF ELIMINATION AFTER CHANGE OF DIET.

A considerable number of experiments upon the time relations of the elimination of nitrogen, sulphur, and phosphorus after the ingestion of proteid food have recently been made in the laboratories of Wesleyan University[a] and in the papers recording the results, the earlier experiments upon this queston are also discussed. The general plan followed in these studies is to place the subject upon a diet and routine similar to that followed in the first series of experiments here recorded, and then to collect and analyze the urine for every three hours, or in some cases every hour and a half, during the day, the night urine being collected in a single nine-hour period. The diet and routine are strictly maintained until the urinary excretion has been found to be practically uniform for two or three days. The subject then takes with breakfast either in addition to the regular food or in place of an isodynamic amount of butter or other food, enough lean beef to furnish the desired amount of extra protein. All

[a] Sherman and Hawk, Amer. Jour. Physiol., 4 (1900), p. 25; Atwater and Hawk, unpublished material; Hawk and Chamberlain, unpublished material.

other conditions remain unchanged and the urine is collected and analyzed by short periods as before, this being kept up until all changes in the urine resulting from the ingestion of the extra food have disappeared. The tests made with different subjects and in different years show slight variations in some details, but the general results agree. Very soon after the ingestion of the extra food there is a rise in the rate of excretion of nitrogen as compared with that found in the corresponding periods of other days. This rise is rapid and the time required to reach a maximum depends upon the amount

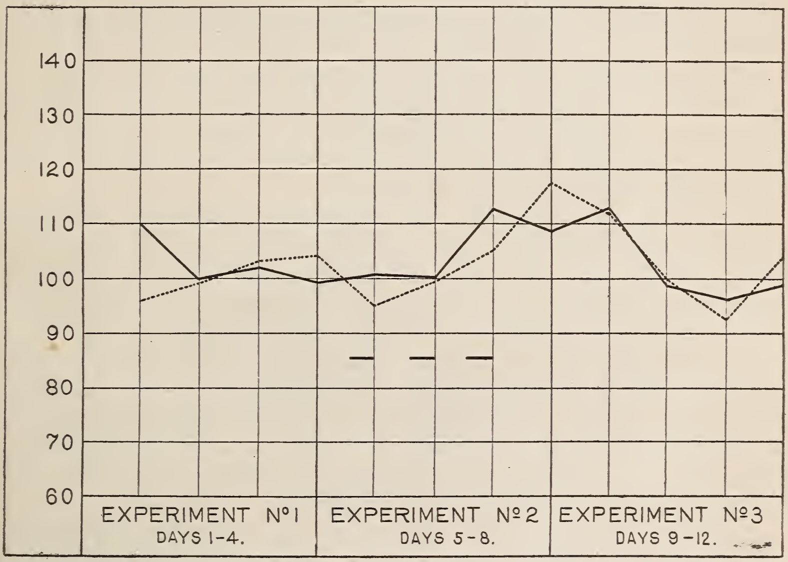

FIG. 1.—Diagram showing fluctuations in the daily excretion of nitrogen and phosphorus during the first series of experiments (Nos. 1–3). In platting these curves the periods of time are represented by the abscissæ, while the ordinates represent the excretions expressed in percentages of the average rate found on the normal days. The short horizontal lines below the curves show the nights in which the losses of sleep occurred.

of extra protein ingested. With 5 grams of extra nitrogen the maximum was reached in from three to four and one-half hours; with 10 grams, generally in from six to nine hours. The fall in the rate of excretion was much less rapid than the rise, but usually the normal rate was regained before the end of the second day. The general features of the curve representing the elimination of the extra nitrogen were the same when the subject was gaining as when he was losing nitrogen and were the same when the beef was simply added to the regular diet as when it was substituted for an isodynamic amount of butter. In general the sulphates and phosphates eliminated were

increased simultaneously with the nitrogen. All of these experiments had to do with the increased elimination brought about by the ingestion of extra protein with a single meal. Some of the present experiments were arranged with a view to studying the "lag" in the elimination when the diet was suddenly changed and the new diet maintained for several days.

On passing from the diet of experiment No. 4 to that of experiment No. 5, there was little change in fuel value, but the amounts of nitrogen and phosphorus ingested were largely increased. Under these conditions there was a "lag" of some days, i. e., some days were required before the rate of elimination become approximately uniform. Experiment No. 4 lasted three days and although experiment No. 5 properly continued but four days, the diet was maintained and the elimination of nitrogen and phosphates determined for an additional four days. The course of the excretion of nitrogen and phosphorus for the eleven days is shown in fig. 2, in which the curves are platted in the same manner as in fig. 1 above.

It will be seen that while the phosphorus elimination reaches a maximum on the third day, the maximum elimination of nitrogen is reached only on the sixth day. It must be noted, however, that the increase of phosphorus in the diet was considerably greater in proportion than the increase of nitrogen, so that although the curves meet on the fifth day it does not follow that equilibrium was then restored. When the elimination of phosphates was at the maximum there was a storage of phosphorus in the body, whereas during the maximum elimination of nitrogen the body was losing that element. The body was in fact nearly in nitrogen equilibrium when the maximum elimination of phosphorus was reached.

In experiments Nos. 7 to 10 the lag was studied under different circumstances from those just described. Instead of attempting to keep the fuel value approximately uniform while changing the amounts of certain constituents, the diet was here kept qualitatively the same, so that every change affected each of the constituents to the same extent. The general outline was as follows: For five days the subject took a restricted diet, which it was thought would be just about sufficient to enable him to do his usual work without becoming uncomfortably hungry. As a matter of fact there was practically no sensation of hunger, but the subject lost during the five days somewhat over 9 grams of nitrogen and about 2 pounds in weight. During a second period of five days twice the original diet was taken. Then the subject returned to the original diet for another period of five days, the object here being to study the lag after a decrease as well as after an increase in the diet. At the end of the third period the diet was again doubled, and the double diet was this time maintained for three days. The results for the eighteen days covered by this series of experiments

are shown in fig. 3, in which the curves are platted in the same manner as in figs. 1 and 2. In this case the sulphate sulphur was also determined, and is represented in the figure by a broken line, nitrogen and phosphorus being represented respectively by solid and dotted lines.

The rises and falls in the curves in this figure on passing from one experimental period to another are not large as compared with the

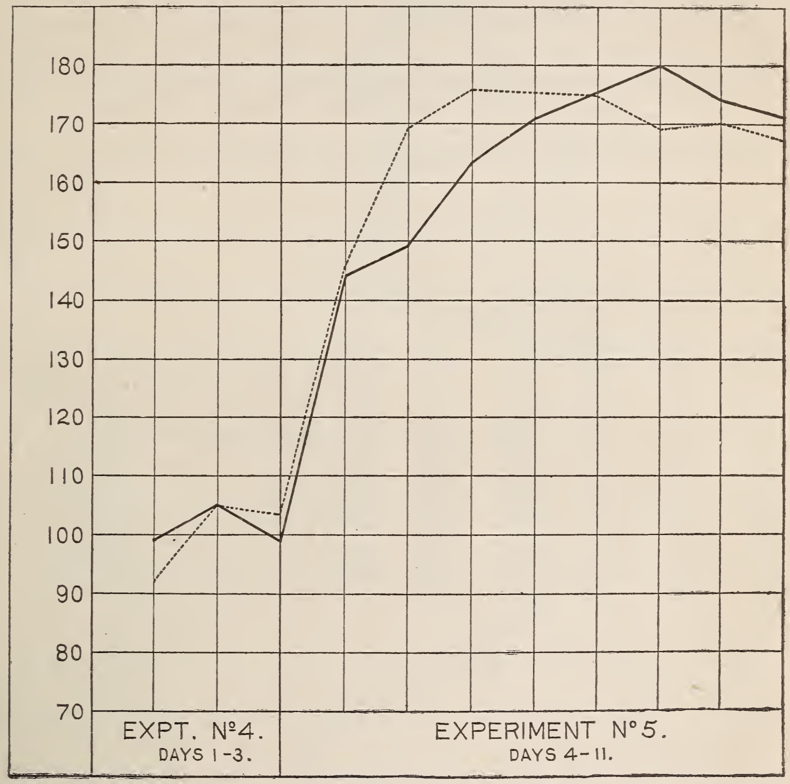

FIG. 2.—Diagram showing fluctuations in the daily excretion of nitrogen and phosphorus during the second series of experiments (Nos. 4 and 5). The curves are platted in the same manner as those in fig. 1.

change in the diet. Thus the diet in experiment No. 8 was twice as great as in experiment No. 7, but the greatest daily elimination was only about one-half larger for nitrogen and sulphur and two-thirds larger for phosphorus. This is mainly because, as would be expected, there was a loss of body material on the small diet and a gain on the large diets. The daily gains and losses are shown in Table 16, the complete balance for each experiment being tabulated beyond.

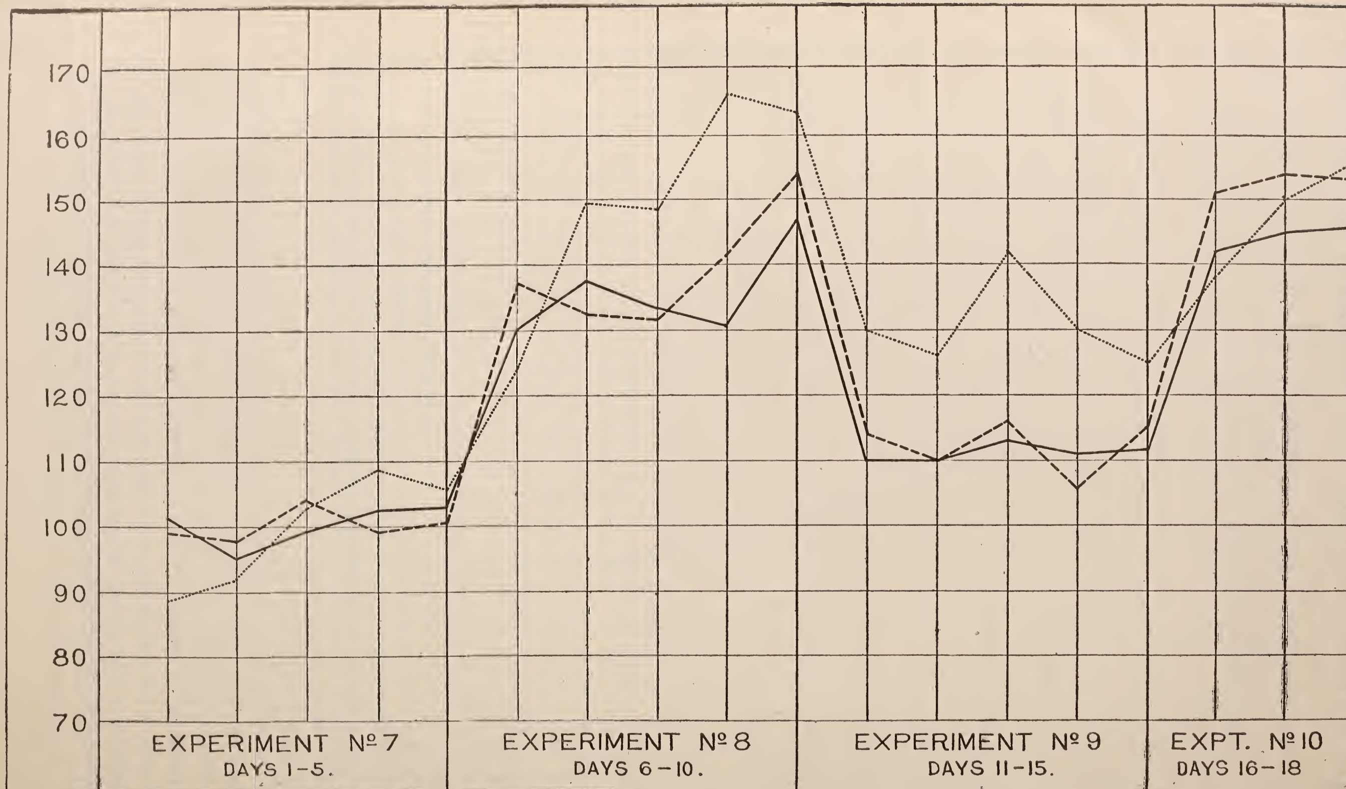

FIG. 3.—Diagram showing fluctuations in the daily excretion of nitrogen, sulphur, and phosphorus during the third series of experiments (Nos. 7-10). The curves are platted in the same manner as those in figs. 1 and 2. The rate of elimination of nitrogen is represented by the solid, that of the sulphur by the broken, and that of the phosphorus by the dotted line.

As has already been stated, the total sulphur and total phosphorus eliminated by the kidneys during each period were determined by analysis of a composite sample of urine. The sulphate sulphur and the phosphorus precipitable by uranium acetate were determined both in the composite sample and in the urine of each day. From the data thus found it is easy to estimate the total sulphur or total phosphorus for the urine of each day if we assume that the ratio of "unoxidized" to total material is constant during the experimental period. While slight errors might result from this assumption, they would be far too small to affect the present discussion. The amounts thus estimated are therefore used in the following table:

TABLE 16.—*Daily gains and losses of nitrogen, sulphur, and phosphorus in experiments Nos. 7–10.*

Experiment number.	Date.	Nitrogen.		Sulphur.		Phosphorus.	
		In urine.	Gain (+) or loss (−).	In urine.	Gain (+) or loss (−).	In urine.	Gain (+) or loss (−).
		Grams.	*Grams.*	*Grams.*	*Gram.*	*Grams.*	*Gram.*
7	July 14–15	11.65	−1.99	0.82	−0.16	0.88	+0.10
	July 15–16	11.12	−1.46	.81	− .15	.92	+ .06
	July 16–17	11.35	−1.69	.87	− .21	1.05	− .06
	July 17–18	11.66	−2	.82	− .16	1.09	− .11
	July 18–19	11.75	−2.09	.83	− .17	1.05	− .07
8	July 19–20	14.83	+4.35	1.04	+ .27	1.23	+ .51
	July 20–21	15.81	+3.37	1.00	+ .31	1.48	+ .26
	July 21–22	15.19	+3.99	1.00	+ .31	1.47	+ .27
	July 22–23	14.91	+4.27	1.08	+ .23	1.65	+ .09
	July 23–24	16.88	+2.30	1.16	+ .15	1.62	+ .12
9	July 24–25	12.64	−2.96	.89	− .23	1.31	− .23
	July 25–26	12.57	−2.89	.86	− .20	1.27	− .19
	July 26–27	13.01	−3.33	.91	− .25	1.43	− .35
	July 27–28	12.73	−3.05	.83	− .17	1.31	− .23
	July 28–29	12.88	−3.20	.90	− .24	1.27	− .19
10	July 29–30	16.23	+3.58	1.19	+ .07	1.39	+ .50
	July 30–31	16.71	+2.94	1.23	+ .03	1.50	+ .39
	July 31–Aug. 1	16.80	+2.85	1.22	+ .04	1.57	+ .32

It will be seen from this table that the gains and losses were considerable, and that equilibrium was not reached in any experiment, even after the continuance of a uniform diet for five days. In view of the large amounts of material gained and lost there is danger that any inferences in regard to lag which could be drawn from these results might be subject to unknown errors arising from the breaking down of body material on the one hand or the transformation of food protein into body protein on the other. It will be shown, however, that, except in cases where the balance was evidently affected by the lag, the proportion of sulphur to nitrogen was nearly the same in the material stored or lost as in the food material actually absorbed. With phosphorus the variations are larger, but a similar relation appears to exist. Hence the question of lag is quite as important here as in the cases where it has been more especially studied, but here it represents not merely the time required for the metabolism of the ingested materials, but to some extent also the time necessary for the body to adapt itself to the increased or decreased diet. On

passing from the insufficient diet of experiment No. 7 to the abundant diet of experiment No. 8 the nitrogen elimination rises on the first day and then remains nearly stationary for three days, during which a large amount of nitrogen is stored, as though the body during these days was replacing the protein previously lost. Then on the fifth day the elimination again arises, though not far enough to establish nitrogenous equilibrium. The elimination of sulphur rises somewhat more sharply on the first day than that of nitrogen, then continues about uniform for two more days, and begins to rise again on the fourth day, continuing to rise on the fifth and reaching a relative rate somewhat higher than that of the nitrogen. The sulphur curve is therefore similar to the nitrogen curve, but the changes are somewhat more marked, and in one case appear to begin earlier. The phosphorus rises sharply during the first and second days and again on the fourth day. What has been suggested with reference to the nitrogen and sulphur appears to be true to a lesser extent of the phosphorus. The increased diet immediately increases the excretion, the increase in this case continuing two days, then for a short period—in this case one day—the elimination is nearly constant, while a considerable proportion is stored in the body, after which the rate of elimination again rises. While the phosphorus does not reach equilibrium as regards income and outgo, it more nearly approaches this condition than either the nitrogen or the sulphur.

When the diet was reduced to one-half, the rate of elimination of each of the three elements studied fell sharply on the first day and showed little if any fall thereafter. Thus, in each case the elimination lagged less in falling than in rising. This is the more striking, in view of the fact that the elimination of both nitrogen and sulphur was rising at the time the change in diet was made.

In the final period, when the double diet was again resumed, the changes in rate of elimination were similar to those found in the first instance, except that the rise shown by the sulphur was somewhat more pronounced. Pressure of other work prevented the continuance of this experiment after the third day.

A somewhat marked but temporary increase in the phosphorus elimination will be noticed on the third day of experiment No. 9, and an examination of the curves shows that simultaneously there occurred a smaller but distinct increase of sulphur, and an increase of nitrogen which relatively is much smaller still and would scarcely have been noticed had only the nitrogen metabolism been studied. Although careful note had been taken of anything which seemed likely to affect metabolism, it is difficult to assign a reason for this increase. During the early parts of the two preceding nights there had been slight restlessness, which was attributed to the warm weather, but previous experiments had indicated that simple loss of sleep, even when very marked, had no great influence upon the metabolism of this subject

and increased the relative amount of phosphorus little, if any, more than that of nitrogen. Such instances as this would seem to lend some support to the view, apparently quite generally held, that the nervous condition of the subject has a greater influence upon the metabolism of phosphorus than upon that of nitrogen. A factor which is perhaps liable to be overlooked in such cases is the influence of the degree of alkalinity of the blood upon the elimination of phosphates through the kidneys.

COMPARISON OF BALANCE OF INCOME AND OUTGO.

It is now generally recognized that the daily balance of income and outgo of nitrogen in the human organism may be influenced by a variety of factors, some of which can not be controlled or even satisfactorily defined. The same is doubtless true of sulphur and probably to a greater extent of phosphorus. In general, it is believed that in the present experiments the metabolism was comparatively free from the influence of such obscure factors, but in interpreting the figures obtained for the balance we must take into account (1) actual errors in the determination of income and outgo, and (2) the elimination on a given day of material whose katabolism is to be attributed to the diet or other conditions of some preceding day or days—in other words, the "lag" in the elimination. Errors in determination of income and outgo fall into two groups—analytical errors, and losses of material. The analytical work was carefully performed by the methods already described. In several cases the constituent sought was present in very small amount, which must have increased the relative errors, and it may be stated that in the opinion of the writer the determinations of sulphur were less satisfactory than those of nitrogen and of phosphorus. As regards the loss of material, it is believed that no appreciable mechanical loss of either food or excretory products could have occurred in any of the experiments, but there may have been larger losses through the perspiration. As the experiments were all made in summer and only one-third to one-half of the ingested water appeared in the urine, considerable quantities of water must have passed through the skin, and more or less loss of the elements studied doubtless occurred in this way. The elimination of nitrogen through the skin has been briefly discussed in a previous bulletin,[a] where it is shown that different estimates of the amount which may be thus lost per day vary from 0.2 gram to 1.36 grams. Very little data seems to be available from which to form an idea of the amounts of sulphur and phosphorus which may have been lost through the skin. Favre[b] found in the perspiration only traces of phosphates, but reported one-fourth as much of alkaline sulphates as of urea, corre-

[a] U. S. Dept. Agr., Office of Experiment Stations Bul. 98, p. 51.

[b] Compt. Rend. Acad. Sci. Paris, **35** (1852), p. 721; Schaffer's Text-book of Physiology, Vol. I, 1898, p. 671.

sponding to an elimination by the skin of about 1 part by weight of sulphur to 8 parts of nitrogen.

Little can be said regarding the amounts of nitrogen and sulphur given off as volatile compounds by the intestine or lost in drying the feces in the air at 100° C. It is known that some nitrogen is thus lost from the feces, probably mainly as ammonia. Loss of ammonia may be avoided by adding acid before drying, but this would result in a loss of sulphur present as sulphids. Hydrogen sulphid is stated to be a normal constituent of the intestinal gases, but the amount of sulphur lost from the body must have been very small in these experiments. It follows from what has been said that, aside from any errors of manipulation or analysis, the figures given for nitrogen and sulphur in urine and feces do not quite represent the total outgo from the body. The average daily balance, as actually determined for each experiment, is given in Table 17.

TABLE 17.—*Balance of income and outgo of nitrogen, sulphur, and phosphorus—average per day.*

Experiment number.	Fuel value of diet per day.	Nitrogen.				Sulphur.				Phosphorus.			
		In food.	In feces.	In urine.	Gain (+) or loss (−).	In food.	In feces.	In urine.	Gain (+) or loss (−).	In food.	In feces.	In urine.	Gain (+) or loss (−).
	Calories.	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gm.*	*Gms.*	*Gm.*	*Gms.*	*Gms.*	*Gms.*	*Gm.*
1	2,908	15.82	0.74	14.31	+0.77	1.12	0.06	0.95	+0.11	2.29	0.78	1.41	+0.10
2	2,901	15.82	.67	14.67	+ .48	1.12	.06	.97	+ .09	2.29	.73	1.48	+ .08
3	2,913	15.82	.70	14.16	+ .96	1.12	.06	.93	+ .13	2.29	.78	1.42	+ .09
4	2,082	12.05	.74	10.43	+ .88	.94	.09	.72	+ .13	1.40	.44	1.03	− .07
5	2,607	18.52	.87	16.50	+1.15	1.44	.08	1.12	+ .24	3.07	1.10	1.74	+ .23
6	1,555	9.76	.27	10.94	−1.45	.69	.02	.75	− .08	1.60	.37	1.12	+ .11
7	1,660	10.11	.45	11.51	−1.85	.70	.04	.83	− .17	1.58	.60	1.00	− .02
8	3,336	20.22	1.04	15.52	+3.66	1.40	.09	1.06	+ .25	3.16	1.42	1.49	+ .25
9	1,656	10.11	.43	12.87	−3.19	.70	.04	.88	− .22	1.58	.50	1.32	− .24
10	3,329	20.70	1.05	16.58	+3.07	1.36	.10	1.21	+ .05	3.26	1.37	1.49	+ .40

The first three experiments show apparent daily gains of one-half to 1 gram of nitrogen and about one-tenth gram of sulphur and phosphorus. These apparent gains may be largely due to the undetermined losses of urea, ammonium salts, sulphates and phosphates through the skin, and of volatile compounds of nitrogen and sulphur through the intestines. In experiment No. 5, in addition to the sources of error just mentioned, we have the effects of "lag" continuing through the experiment, as explained above. Experiment No. 6 shows a moderate loss of nitrogen and a corresponding loss of sulphur, but a slight apparent gain of phosphorus. In this case, however, the elimination of phosphorus (as also of nitrogen and sulphur) by the intestine is relatively so small as to mark the experiment as somewhat exceptional.

It may be said that in these six experiments the sulphur balance follows that of nitrogen, but in no case is the gain or loss great enough to justify calculations of the composition of the material stored or broken down.

In experiments Nos. 7, 8, and 9 the gains and losses are larger, and here it is probably safe to draw inferences regarding the composition of the material stored or lost by the body, though the undetermined errors already discussed will of course affect the accuracy of such deductions. Neglecting these errors, the figures given for "balance" would indicate that in the material lost in the first of these experiments the ratio of sulphur to nitrogen was as 1:10.9, in that stored in the second as 1:14.6, and in that lost in the third as 1:14. The ratio in the food consumed was 1:14.4, in the food material actually absorbed (food minus feces) it was 1:14.6. It was calculated above that this ratio in serum globulin is 1:14.3, in myosin 1:13.1, in serum albumin 1:7.1, and in the glycoproteids of connective tissue and of bone as about 1:5. Thus the ratio is narrower in the tissue proteids generally than in the food here consumed, but in serum globulin the ratio is about the same, and in myosin not greatly different. A strict interpretation of these ratios would thus lead to the conclusion that in the first period the body metabolized the food eaten and some of its own material in which the ratio is narrower; that in the second, a part of the protein of the food is either stored without essential change or converted for storage into some form of body protein in which the ratio is practically the same (as in the case with serum globulin), and that the proteid lost in the third period was of the same nature as that stored in the second period. Such a method of interpretation is in line with that followed by Kolpatcka in his studies upon dogs, but for the reasons already given it is believed that such conclusions must be accepted with reserve until more is known of the conditions influencing the "balance" and the "lag." These experiments, however, do at any rate strongly emphasize the close parallelism between the metabolism of nitrogen and that of sulphur when the diet is normal and is continued uniform for a period long enough to practically eliminate the effects of the lag. In experiment No. 10, which continued but three days, the balance is much influenced by the lag, so that in this case the apparent gains show no relation to the proportions of the two elements in the food.

On the other hand the phosphorus metabolism does not show such a close parallelism to the metabolism of nitrogen. In experiment No. 7, where the loss of nitrogen was nearly constant throughout, there was at first a slight gain and later a slight loss of phosphorus, the net result being an almost perfect balance. This is probably due to the comparative richness of the diet in phosphorus, so that it supplied sufficient of this element for the needs of the body, while the protein of the diet was so far insufficient as to result in considerable loss of nitrogen and sulphur. During the five days of abundant diet (experiment No. 8) there was a storage of 1.25 grams of phosphorus, and almost exactly the same amount was given up during the following five days of restricted diet. The three days of experiment No. 10

show a large apparent gain of phosphorus, but this is largely due to the "lag" and can not be considered to represent permanently-stored material.

Any comparison of the nitrogen and phosphorus metabolism in the human organism is complicated by the varying proportions of phosphorus eliminated in the feces. Thus in experiments Nos. 8 and 9 the diet was qualitatively the same, yet in the former 44.9 per cent and in the latter only 31.6 per cent of the phosphorus in the food was found in the feces. Two explanations suggest themselves, (1) that only a part of the phosphates from body katabolism may appear in the urine, the remainder being eliminated through the intestine, as in the herbivorous animals; (2) that the proportion which the body absorbs may depend not only upon the nature of the ingested phosphates, but also upon the condition and needs of the body. The former is probably true to some extent, but it seems probable that the latter also operates in some cases, as in that just mentioned, where, on doubling the diet, a much smaller proportion of the phosphorus present was assimilated.

The larger proportion of ingested phosphorus which appears in the feces makes the proper separation of the latter a much more important matter in experiments in which the balance of phosphorus is to be determined than in those in which only nitrogen or nitrogen and sulphur are studied.

In view of the results which have recently been obtained upon the assimilation of the phosphorus of casein,[a] the phosphates found in the feces in these experiments should probably be attributed mainly to the calcium phosphate of the food. It should be remembered also that in the present experiments the diet was unusually rich in phosphates, and the proper interpretation of the results must await the completion of similar experiments upon different diets.

The experiments here reported afford no data for a direct comparison of the nutritive values of different proteids, the food materials used having been similar through the whole series. However, in view of the recent work upon the nutritive value of the proteids of milk,[b] it is interesting to note the tendency shown in experiments Nos. 1 to 4 to store protein on a diet considerably smaller than that usually estimated for a subject of vigorous appetite and doing a considerable amount of work.

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[a] Marcuse, Arch.Physiol. [Pflüger]. **67** (1897), p. 373; Knopfelmacher, Wiener Klin. Wchnschr., **12** (1899), p. 1308; Nicko, Ztschr. Biol., **39** (1900), p. 430; Müller, Ibid., p. 451.

[b] Among the many recent papers may be noted: Marcuse, Arch. Physiol. [Pflüger], **64** (1896), p. 223; Steinitz, Ibid., **72** (1898), p. 75; Rohmann, Berl. Klin. Wchnschr., **35** (1898), p. 789; Albu, Fortschr. Med., **17** (1899), p. 505; Poda and Prausnitz, Ztschr. Biol., **39** (1899–1900), p. 279.

SUMMARY.

The digestibility of the protein of the bread and milk diet as found in nine of the ten experiments agreed closely with the results calculated, assuming 97 per cent as the coefficient for milk and 90 per cent as that for bread. The digestibility was not appreciably influenced by loss of sleep nor by the continuance of the diet for twelve or eighteen days.

The proportions of protein digested from a restricted diet were about 0.7 per cent higher than those digested from a liberal diet of the same composition.

Marked loss of sleep for three successive nights resulted in a small increase in the amounts of nitrogen, sulphur, and phosphorus excreted. The increase of sulphur was proportional to that of nitrogen and the increase of phosphorus was very slightly larger, the relative difference being no greater than might be attributed to the usual daily variations.

The increased elimination resulting from loss of sleep did not appear until the third day, while changes resulting from alteration of the diet were always perceptible on the first day.

The data collected regarding the relative "lag" of nitrogen, sulphur, and phosphorus are not yet sufficient to permit general conclusions to be drawn.

In general the metabolism and "balance" of sulphur ran approximately parallel with that of nitrogen.

The renal elimination and "balance" of phosphorus showed fluctuations similar to those of nitrogen, but not so closely parallel as in the case of sulphur. The elimination of phosphorus by the intestine was large and variable, making the accurate separation of the feces an important factor in the determination of the phosphorus balance.

The above statements are intended merely to summarize the results of the experiments here reported. As these were all made upon a single subject and with only two or three food materials, it would obviously be unsafe to generalize broadly from the results. As already explained, the work was undertaken not so much with a view to obtaining results of intrinsic interest as to get data regarding methods of work and possible sources of error, and thus facilitate the study of the sulphur and phosphorus metabolism in connection with certain of the series of nutrition investigations to which the present experiments belong.

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